Evans, et al.

U.S.S.N.: Filed:

09/786,009 April 17, 2001

Page

2

- 13. The method according to claim 12, wherein the intein is selected from Sce Vma and Mxe Gyr A.
 - 14. The method of claim 12, wherein the thiol reagent is selected from 2-mercaptoethanosulfonic acid, thiophenol, dithiothreitol, and 3-mercaptopropionic acid.
 - 15. The method according to claim 12, wherein the precursor protein is selected from a Bst DNA polymerase I large fragment, Thioredoxin and a cytotoxic protein.
 - 16. The method according to claim 12, wherein the precursor protein is selected from a maltose binding protein and paramyosin.
 - 17. A method for expressing a protein precursor, comprising: preparing a plasmid having a multiple cloning site between two restriction endonuclease recognition sites; and

inserting a protein encoding nucleic acid sequence into the plasmid upstream of an intein encoding nucleic acid sequence, wherein the cleavage agent sequence is optionally upstream to a binding protein encoding nucleic acid sequence.

18. The method of claim 17, wherein the binding protein encoding nucleic acid sequence is chitin binding protein encoding mucleic acid sequence.



21008

Evans, et al.

U.S.S.N.: Filed:

09/786,009 April 17, 2001

Page

3

- 19. The method according to claim 17, wherein the multiple cloning site contains a linker sequence.
- 20. The method according to claim 19, wherein the linker sequence is selected from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:4.
- 21. The method according to claim 17, wherein the plasmid is a pTXB plasmid.
- 22. A method of ligating a synthetic fragment in vitro to an inactive expressed protein so as to restore protein activity, comprising:
- (a) expressing an inactive truncated form of the protein linked to a thiol inducible cleavage agent; and cleaving the protein in the presence of a thiol reagent so as to form an expressed protein with a C-terminal thioester;
- (b) preparing a synthetic peptide having an N-terminal cysteine;and
- (c) ligating the inactive form of the protein with the synthetic peptide to restore protein activity.
- 23. The method of claim 20, wherein the protein is a cytotoxic protein.
- 24. The method of claim 21, wherein the cytotoxic protein is a restriction endonuclease.